WEST Search History

updated 3/03

DATE: Monday, March 10, 2003

Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT; PLUR=YES; OP=AND		
L1	platelet near5 surface	2548	L1
L2	L1 near10 sugars	1	L2
L3	L1 near5 glucose	1	L3
L4	L1 near5 moiet$	7	L4
L5	L1 same antigens not l2 not l3 not l4	129	L5
L6	L1 near10 antigens not l2 not l3 not l4	69	L6
L7	L1 near10 glyco$	253	L7
L8	platelet near5 heat	174	L8
L9	L8 and blood	50	L9

END OF SEARCH HISTORY

L6: Entry 1 of 69 File: USPT Mar 19, 2002

DOCUMENT-IDENTIFIER: US 6358707 B1
TITLE: Human F11 antigen: a novel cell surface receptor involved in platelet aggregation

Brief Summary Text (6):
Platelets and platelet membrane glycoproteins play a significant role in inflammatory and thrombotic reactions. Specific anti-platelet autoantibodies and alloantbodies to membrane glycoproteins, such as GPIb, GPIIb, and GPIIIa, among others have been identified in patients with clinical disorders like drug-dependent thrombocytopenia purpura, post transfusion purpura, septicemia, neonatal isoimmune thrombocytopenia, and chronic immune thrombocytope, among others (Woods, et al. 1984a Blood, 64:156-160; Woods, et al. 1984b, Blood, 63: 368-375; Kickler, et al. 1988 Blood 71: 894-898; Christie, et al. 1987 Br. J. Haematol, 67: 213-219). Autoantibodies against such cell surface antigens interact with platelets and cause platelet aggregation. Identification of these self antigens has helped elucidate the molecular mechanism underlying the cell activation. Several auto-antibodies activate platelets either via an FcgRII (CD32) receptor mediated process (Rubinstein, et al. 1991, J. Immunol. 147: 3040-3046; Horsewood, et al. 1991, Blood 78:1019-1026) or as F(ab')2 fragments without the necessity of an intact IgG molecule. Korneck, et al. in 1991 (J. Biol. Chem. 265: 10042-10048) had previously reported a monoclonal antibody, mAb F11 which recognized two membrane proteins of 32 and 35 kd, termed the F11 antigen. Partial peptide sequencing of the purified F11 antigen was also reported (Naik, et al. 1995 Biochem. J., 310: 155-162) and its ability to induce vesicular secretion and aggregation in human platelets has been studied.

Brief Summary Text (7):
The F11 antigen is a novel platelet membrane surface glycoprotein which is cross-linked with the FcgRII receptor when platelets are activated by the stimulatory mAb F11. While the physiological and pathophysiological significance of F11 antigen remains to be completely explored, such proteins clearly represent putative targets for therapeutic intervention in a wide range of distinct pathological, inflammatory and thrombotic conditions.

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L6: Entry 5 of 69 File: USPT Aug 28, 2001

DOCUMENT-IDENTIFIER: US 6280932 B1
TITLE: High affinity nucleic acid ligands to lectins

Detailed Description Text (372):
Since the P-selectin ligands were isolated against purified protein, their ability to bind P-selectin presented in the context of a cell surface was determined in flow cytometry experiments with activated human platelets. Platelets were gated by side scatter and CD61 expression. CD61 is a constitutively expressed antigen on the surface of both resting and activated platelets. The expression of P-selectin was monitored with anti-CD62P monoclonal antibody (Becton Dickinson). The mean fluorescence intensity of activated platelets, stained with biotintylated-PF377s1 (SEQ ID NO: 223)/SA-PE (Example 27, paragraph G), is 5 times greater than that of similarly stained resting platelets. In titration experiments half maximal fluorescence occurs at approximately 50 pM PF377s1 (EC50) which is consistent with its equilibrium dissociation constant, 60 pM, for PS-Rg. Binding to platelets is specific by the criterion that it is saturable. Saturability has been demonstrated not only by titration but also by competition with unlabeled PF377s1.

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L6: Entry 6 of 69 File: USPT Jun 12, 2001

DOCUMENT-IDENTIFIER: US 6245782 B1
TITLE: Methods of inhibiting platelet activation with selective serotonin reuptake inhibitors

Other Reference Publication (9):
Serebruany, V.L., et al., "Crossreactivity of Human versus Swine Platelet Surface Antigens is Similar for Glycoproteins Ib and IIIa, but Not for the Glycoprotein Iib/IIIa Complex," J. Thromb. Thrombolysis, 5(1): 37-41 (1998).

L6: Entry 11 of 69 File: USPT Nov 30, 1999

DOCUMENT-IDENTIFIER: US 5993804 A
TITLE: Pharmaceutically acceptable fixed-dried human blood platelets

Detailed Description Text (33):
The markers employed in the tests described in Table 3, CD62 and GP53, are commercially available antibodies purchased from Becton-Dickinson, Inc. and are used to indicate the presence on the surface of the platelet of antigens released from platelet granules. The presence of granule-released antigens on a platelet surface is taken as evidence of platelet activation. Antibodies against these antigens are referred to as activation markers. Antibodies against Platelet-derived growth factor (PDGF) are used to detect platelet membrane bound PDGF. The PDGF antibody was purchased from Genzyme (Cambridge, Mass.). The vessels used in the Baumgartner adhesiveness tests were obtained from a normal dog.

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L6: Entry 12 of 69 File: USPT Aug 17, 1999

DOCUMENT-IDENTIFIER: US 5939524 A
TITLE: Platelet GPIII P1.sup.A1 and P1.sup.A2 epitopes, their preparation and use

Brief Summary Text (3):
The present invention relates to the platelet surface glycoprotein referred to as GPIIIa, and more particularly to tertiary structure-dependent antigen denominated Pl.sup.A that is part of the GPIIIa molecule, their preparation and use.

L6: Entry 22 of 69 File: USPT Jun 30, 1998

DOCUMENT-IDENTIFIER: US 5773228 A
TITLE: Activation-dependent protein expressed on the surface of activated platelets and antibodies thereto

Brief Summary Text (7):
The identification of other antigens specifically expressed on the surface of activated platelets may help elucidate some of the molecular changes that occur during platelet activation, particularly those responsible for changing the platelet from a quiescent cell to a fully adherent thrombocyte. Perhaps the best example of information derived from the study of platelet surface proteins is the research on the glycoproteins IIb/IIIa. Studies of the GPIIb/IIIa glycoproteins, using specific ligands and monoclonal antibodies, are beginning to elucidate the molecular rearrangements that occur in the GPIIb/IIIa complex, with regard to conformation, density, etc., which are responsible for its conversion to a fully competent "receptor" that mediates platelet aggregation (reviewed in Bennett, J.S., Semin. Hematol. 27:186-204 (1990)). Although these studies have significantly enlarged our grasp of the mechanisms of platelet aggregation, our understanding of other platelet events remains less complete.

Detailed Description Text (68):
Hybridomas which produced antibody that specifically bound to rabbit platelets were fully tested to determine whether their binding to platelets was inhibited by plasma proteins. One of these hybridomas, 12A7, bound to activated platelets and was not significantly inhibited by plasma. Comparative whole platelet binding experiments were performed to determine whether the antigen, recognized by MAb 12A7, was differentially expressed by resting and activated platelets. Purified, radioiodinated MAb 12A7 was incubated with resting and thrombin-activated platelets (FIG. 3). When 12A7 was incubated with resting platelets (3.6.times.10.sup.7 cells), there was minimal, if any, antibody binding. In comparison, when 12A7 was incubated with thrombin-activated platelets (5.times.10.sup.6 cells), there was a marked increase in the amount of antibody bound. This suggested that MAb 12A7 recognized an activated platelet protein (TAPP-2) or antigen that was chiefly expressed on the platelet surface after cellular activation by thrombin.

Detailed Description Text (77):
We have generated a monoclonal antibody, 8B6, to a novel protein antigen present on the surface of thrombin-activated platelets. This thrombin-activated platelet protein (TAPP-1) was immunopurified and amino terminal amino acids were sequenced. It was found to contain a hitherto undescribed amino acid sequence. From SDS-PAGE analysis, TAPP-1 appears to be a complex of M.sub.r .about.250 kd. Preliminary data suggested that TAPP-1 was composed of 3 chains which are disulfide linked. One chain of .about.138 kd appears from its staining pattern to be a glycoprotein. The next largest chain(s) consist of a triplet at an average M.sub.r .about.56 kd. The smallest chain appeared t be .about.42 kd.

Detailed Description Text (83):

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L11: Entry 26 of 68 File: USPT Nov 10, 1998

DOCUMENT-IDENTIFIER: US 5833991 A
TITLE: Glycine-containing sequences conferring invisibility to the immune system

Detailed Description Text (91):
A chimeric gene encoding a recombinant protein of the invention comprising the influenza matrix protein 1 and a glycine-containing sequence is introduced and expressed in HLA A2 positive fibroblasts, EBV transformed lymphoblastoid cells lines (LCL), or mitogen-induced blasts according to gene delivery methods described herein. Chimeric gene expression is confirmed by immunofluoresence using an antibody specific for influenza matrix protein 1 and an antibody specific for the glycine-containing sequence. Chimeric gene expression also may be confirmed via Western blotting, according to standard procedures. A preparation comprising host cells expression the transduced chimeric gene is used as a target population in a cytotoxicity assay. Cytotoxic effector cells may be a polyclonal CTL population from an HLA A2 positive individual stimulated with autologous influenza A virus-infected cells or CTL clones specific for the 58-66 epitope, as described (Morrison et al., Eur. Jour. Immunol. 22:903, 1992).

06592089 90293226 PMID: 2358549

Light microscope study of the coexistence of GABA-like and glycine-like immunoreactivities in the spinal cord of the rat.

Todd A J; Sullivan A C
Department of Anatomy, University of Glasgow, United Kingdom.

Journal of comparative neurology (UNITED STATES) Jun 15 1990, 296 (3) p496-505, ISSN 0021-9967 Journal Code: 0406041
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

The distributions of GABA-like and glycine-like immunoreactivities in the rat spinal cord were compared by using postembedding immunohistochemistry on semithin sections. In laminae I, II, and III, the proportions of GABA immunoreactive cells were 28%, 31%, and 46%, respectively, whereas for glycine immunoreactive cells the proportions were 9%, 14%, and 30%. Nearly all of the glycine immunoreactive cells in this area were also immunoreactive with the anti-GABA antiserum. In lamina II, some Golgi-stained islet cells were glycine immunoreactive, whereas others were not. Immunoreactive cell bodies were also present in the remainder of the grey matter. Some of these reacted with anti-GABA or **antiglycine** antiserum; others showed immunoreactivity with both antisera. Immunoreactive axons were found in the ventral and lateral funiculi of the white matter. Many large axons reacted with **antiglycine** antiserum, whereas GABA-immunoreactive axons were mostly of small diameter. Some large and small axons showed both types of immunoreactivity. These results suggest that the inhibitory neurotransmitters GABA and glycine coexist within cell bodies and axons in the rat spinal cord.

Tags: Animal; Male
Descriptors: *Glycine--metabolism--ME; *Spinal Cord--metabolism--ME; *gamma-Aminobutyric Acid--metabolism--ME; Immunohistochemistry; Rats; Spinal Cord--cytology--CY
CAS Registry No.: 56-12-2 (gamma-Aminobutyric Acid); 56-40-6 (Glycine)
Record Date Created: 19900731

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10mar03 09:49:00 User228206 Session D1931.2
$1.04 0.326 DialUnits File155
$0.63 3 Type(s) in Format 9
$0.63 3 Types
$1.67 Estimated cost File155
$0.22 TELNET
$1.89 Estimated cost this search
$1.89 Estimated total session cost 0.482 DialUnits

Status: Signed Off. (1 minutes)

01241645 JICST ACCESSION NUMBER: 91A0448640 FILE SEGMENT: JICST-E
Glycine as a neurotransmitter in the nervous system. A study with anti - glycine antibody .
SENBA REIJI (1); AOKI HIDEKO (1); KASHIWAMATA SHIGEO (1)
(1) Aichi Prefect. Colony, Inst. for Developmental Res.
Cell, 1991, VOL.23,NO.5, PAGE.147-150, FIG.3, REF.7
JOURNAL NUMBER: F0692AAM ISSN NO: 0386-4766
UNIVERSAL DECIMAL CLASSIFICATION: 591.18.05+591.48
LANGUAGE: Japanese COUNTRY OF PUBLICATION: Japan
DOCUMENT TYPE: Journal
ARTICLE TYPE: Commentary
MEDIA TYPE: Printed Publication
DESCRIPTORS: neurotransmitter; nerve cell; nerve fiber; auditory sense; retina; antibody; amino acid metabolism; chemoreceptor; amino acid; aliphatic amine; aliphatic carboxylic acid
BROADER DESCRIPTORS: cell(cytology); neuron; nerve tissue; animal tissue; biomedical tissue; organization; sense; optic organ; sense organ; histomembrane; membrane and film; metabolism; receptor; amine; carboxylic acid
CLASSIFICATION CODE(S): EJ12010J

1/9/3 (Item 2 from file: 94)
DIALOG(R)File 94:JICST-EPlus

00385286 JICST ACCESSION NUMBER: 87A0161117 FILE SEGMENT: JICST-E
Immunocytochemical study of glycine in rat brain and spinal cord with purified anti - glycine antibody .
AOKI HIDEKO (1); SENBA REIJI (1); KASHIWAMATA SHIGEO (1)
(1) Aichikenshinshinshogaishakoroni Hattatsushogaiken
Shinkei Kagaku(Bulletin of the Japanese Society for Neurochemistry), 1986, VOL.25,NO.1, PAGE.466-468, FIG.5, REF.12
JOURNAL NUMBER: Y0225AAP ISSN NO: 0037-3796
UNIVERSAL DECIMAL CLASSIFICATION: 591.18.05+591.481
LANGUAGE: Japanese COUNTRY OF PUBLICATION: Japan
DOCUMENT TYPE: Conference Proceeding
ARTICLE TYPE: Original paper
MEDIA TYPE: Printed Publication
DESCRIPTORS: rat; brain; spinal cord; enzyme antibody technique; tissue distribution; sensory nerve; motor nerve; amino acid; aliphatic amine; aliphatic carboxylic acid
BROADER DESCRIPTORS: Myomorpha; Rodentia; Mammalia; Vertebrata; animal; central nervous system; nervous system; labeled antibody method; immunoassay; bioassay; distribution; peripheral nerve; amine; carboxylic acid
CLASSIFICATION CODE(S): EJ12030F

4,727,023 [IMAGE AVAILABLE] Feb. 23, 1988 L1: 1 of 1

Preparations for use in solid phase immunoassays comprising monoclonal antibodies covalently embedded in their immobilized hybridoma cells

INVENTOR: Lynn Wang, Jerusalem, Israel
Michael Inbar, Mazkeret Batya, Israel
ASSIGNEE: I.D.L. International Diagnostic Laboratories Limited, Jerusalem, Israel (foreign corp.)

APPL-NO: 06/773,931
DATE FILED: Sep. 9, 1985
FRN-PRIOR: Israel 72924 Sep. 12, 1984
INT-CL: [4] G01N 33/53; G01N 33/558; C12N 15/00
US-CL-ISSUED: 435/7; 424/1.1; 435/172.2, 174, 240.27; 436/519, 547, 548, 804, 535; 935/110
US-CL-CURRENT: 435/7.23; 424/1.49; 435/7.24, 172.2, 174, 968; 436/519, 535, 547, 548, 804; 935/110
SEARCH-FLD: 435/182, 243, 260, 7, 172.2; 436/519, 535, 547; 424/1.1

REF-CITED:

U.S. PATENT DOCUMENTS

4,409,331	10/1983	Lim	424/93

OTHER PUBLICATIONS

Taber's Cyclopedic Med. Dictionary (1973), Davis Co., Phila., Pa., p. F-25.

ART-UNIT: 128
PRIM-EXMR: Sam Rosen
LEGAL-REP: Browdy and Neimark

ABSTRACT:
A reagent for use in solid phase immunoassay diagnostics comprises a matrix of non-active hybridoma cells embedded with its self-produced, covalently bound, actively presented monoclonal antibodies.
The solid phase reagent according to the invention is prepared by incubating in vitro a culture medium containing active hybridoma cells capable of producing monoclonal antibodies, allowing the formation of antibodies to proceed, separating and washing said cells, resuspending the cells in a buffer solution, adding to the resulting suspension an inactivator substance capable of converting active hybridoma cells into the non-active state.

13 Claims, 8 Drawing Figures
EXMPL-CLAIM: 1,8
NO-PP-DRAWING: 8

SUMMARY:

The present invention concerns monoclonal antibody preparations for use in solid phase immunoassays.

It has been known for some time (see George Kohler and Ceasar Milstein, ((1975) Nature, 256, 465) that hybridoma cell lines can be used for the production of monoclonal antibodies (McAb). The parents of such hybridomas are, on the one hand, myeloma cells and, on the other hand, lymphocytes such as spleen cells obtained from immunized animals, e.g. mice, and selected for their ability to produce a particular McAb. Accordingly, by definition hybridomas are hybrid-myelomas which have inherited from the parent myeloma the ability to grow in vitro and in vivo and from the parent lymphocyte the ability to produce monoclonal antibodies of a desired specificity.

Conventionally, monoclonal antibodies can be used for solid phase immunoassays when they are chemically combined with macro-molecular carrier substances such as polymers. This method has, however, the shortcoming that by the chemical combination of the monoclonal antibody with a carrier substance there occurs a chemical modification which may affect unfavourably the activity of the antibodies and the performance of the immunoassay. Also, the recovery of the monoclonal antibodies from the growth medium and the subsequent chemical reaction, are both time-and material-consuming operations.

In the following specification and claims the terms "active" and "non-active" will be used in conjunction with hybridoma cells. The term "active" is meant to signify a viable hybridoma cell culture that is growing and actively producing monoclonal antibodies. The term "non-active" is used to signify a killed hybridoma cell/monoclonal antibodies complex which no longer produces new antibody molecules nor retains any cell division capability.

In accordance with the present invention there is provided a reagent for use in solid phase immunoassay diagnostics comprising a matrix of non-active hybridoma cells which are embedded with and convalently bound to its self-produced, actively presented monoclonal antibodies.

If desired, the solid phase reagent according to the invention may also contain auxiliary substances for use in immunoassay such as a dye or colour indicator for colorimetric tests, enzymatic, fluorescent, luminescent or radioactive labelling materials, etc.

The solid reagent according to the invention may for example, be in the form of a powder, beads, pellets, tablets, aggregations and the like. It may, morever, be mounted on solid supports such as sticks, strips, tubes, microplates, etc.

The invention also provides a process for the preparation of a solid reagent for use in diagnostic immunoassay, comprising incubating in vitro a culture medium containing active hybridoma cells capable of producing monoclonal antibodies, allowing the formation of antibodies to proceed, separating and washing said cells, resuspending the cells in a buffer solution, adding to the resulting suspension a fixture capable of converting active hybridoma cells into the non-active state (inactivator substance), thereby to produce an immobilized non-active hybridoma cells/monoclonal antibodies complex, and binding excessive free inactivator substance.

In order to inactivate the hybridoma cells to form the non-active, immobilized complex, various reagents may be used, such as bi- or multi-functional crosslinking reagents. Among others, aliphatic aldehydes have been found suitable, such as for example, glutaraldehyde.

The solid phase reagent according to the invention can be stored for long periods of time and used when required in assay systems such as radioimmunoassay (RIA), enzyme immunoassay (EIA), fluorescent immunoassay (FIA), luminescence immunoassay (LIA), colorimetric immunoassay, cytochemistry and cell sorting, agglutination, etc.

The solid phase McAb reagent according to the invention is based on a new concept of using the hybridoma cells themselves as a component of the assay system, which has never been proposed before. This has the advantage over known solid phase McAb reagents in that the antibodies are

in their native form and in high concentration and accordingly, the reagent according to the invention is considerably more reliable and accurate than known solid phase McAb reagents. Also, the production of the reagent according to the invention is simpler and cheaper than the production of conventional solid phase McAb preparations.

In principle, for using a McAb reagent according to the invention for the determination of an antigen, the sample in which the amount of antigen is to be determined is incubated with the reagent, for example, together with a labelled antigen probe. The unlabelled antigen in the sample competes with the labelled antigen for the combination site of the McAb on the immobilized non-active hybridoma cells. The resulting antibody-antigen complex bearing cells are separated from their liquid environment by a single step following which the amount of labelled antigen in the complex is determined. This amount is inversely proportional to the quantity of antigen originally present in the sample. A relationship can be constructed using known amounts of antigens in control samples. Thus the concentration of the antigen in the unknown specimen can be calculated.

In some instances, a combination of a certain type of labelled antigen probes together with special instrumentation allow for the determination of antigen in the unknown sample in a one-step homogeneous immunoassay using the solid phase McAb reagent according to the invention. For example, the antigen to be determined in the sample is incubated with the McAb reagent together with a fluorescein-labelled antigen probe. The unknown antigen competes with the fluorescence-labelled antigen for the reaction sites on the McAb on the immobilized non-active hybridoma cells. The entire reaction mixture can then be analyzed by Fluorescence Flow Cytometry without further manipulations. As this type of instrument measures and records the fluorescent intensity of each single cell, resulting in an integrated fluorescence distribution profile of the entire population of cells in the reaction mixture, the amount of mean fluorescence found on these immobilized hybridoma cells according to the invention will be inversely proportional to the quantity of unknown antigen in the sample.

DRAWING DESC:

The invention will now be described with reference to the following examples and annexed drawings which are graphical representations showing the following:

FIG. 1--Specifity of binding of .sup.3 H-propranolol to a solid phase McAb reagent according to the invention;

FIG. 2--Dose response of an anti-propranolol solid phase McAb reagent according to the invention;

FIG. 3--Inhibition of the binding of .sup.3 H-propranolol to an anti-propranolol solid phase McAb reagent according to the invention by means of native free anti-propanolol McAb;

FIG. 4--Specificity of the binding .sup.125 I-digoxin by an anti-digoxin solid phase McAb reagent according to the invention;

FIG. 5--A standard curve of RIA assay for digoxin with an anti-digoxin solid phase McAb reagent according to the invention;

FIG. 6--Inhibition of the binding of .sup.125 I-digoxin with solid phase anti-digoxin McAb reagent according to the invention by means of a native free anti-digoxin McAb.

FIG. 7--Fluorescence flow cytometry curves of fixed B-20 cells. Unlabeled B-20 cells (left) and B-20 cells incubated with FITC-HSA (right). The arrow indicates the reference point described in the test.
FIG. 8--Dose response curve of HSA using fixed B-20 cells, FITC-HSA and unlabeled HSA. Experiments were carried out on FACS and data summarizes 3 separate experiments as indicated by different symbols.

DETDESC:

EXAMPLE 1. Preparation of non-active Hybridoma Cells - McAb Complex

Actively growing hybridoma cells were harvested by centrifugation and washed with serum free medium. The cells were resuspended in phosphate-buffer-saline (10 mM sodium phosphate pH 7.4, 150 mM sodium chloride) at 10.sup.7 cells per ml. Electronmicroscopically grade glutaraldehyde added to a final concentration of 2.5% (v/v). The reaction mixture was incubated at 4.degree. C. for 30 minutes and glycine was added to a final concentration of 1M. After incubation for 10 minutes at 4.degree. C. the suspension was centrifuged at 1200 RPM at 4.degree. C. for 10 minutes and the cell pellet washed once with phosphate-buffered saline.

The cell suspension was adjusted to a concentration of 10.sup.7 cells per ml in phosphate-buffered saline (PBS) containing 0.1% sodium azide and stored at 4.degree. C. for further use.

EXAMPLE 2

Specific immuno-recognition of radiolabelled propranolol to an anti-propranolol solid phase McAb reagent according to the invention

Hybridoma P-28: a cloned cell line producing monoclonal anti-propranolol antibodies, Hybridoma Dig 18: a cell line secreting monoclonal anti-digoxin antibodies; and NS1: the parental mouse myeloma cell line used in the creation of the hybridomas and which by itself does not secrete any complete immunoglobulin molecules, were each fixed as described in Example 1. Radioactive propranolol (4-.sup.3 H-propranolol, Amersham, specific activity=20 curies per m mole) at a concentration of 0.5 p mole or 150 pg per 0.1 ml per reaction was incubated with different amounts of the glutaldehyde fixed cells (0.1 ml) at room temperature for 1 hour. The mixtures were centrifuged at 3000 RPM at 4.degree. C. for 20 minutes, the supernatants aspirated, and the cell pellet washed once with 1 ml of cold PBS. The washed cells were lysed in a total of 0.3 ml 0.1N NaOH and transferred to 3 ml of Pico-Fluor 30 scitillation counting solution. Radioactivity was measured with a Tricard Scillilation Counter.

As shown in FIG. 1, only hybridoma P-28, the cell line that produced monoclonal antibodies to propranolol, binds specifically the radioactive antigen in a linear fashion, whereas both the McAb producing but non-relevant cell line (Dig 18) and the NS1 myeloma cells did not bind the labelled antigen probe.

EXAMPLE 3

Propranolol immunoassay using an anti-propranolol solid phase McAb

reagent according to the invention

A pre-determined amount of an anti-propranolol solid phase McAb prepared by the procedure of Example 1 (2.times.10.sup.5 cells per 0.2 ml), was incubated with different concentrations of propranolol dissolved in human serum (0.1 ml) for 1 hour at 37.degree. C. .sup.3 H-propranolol was added at 150 pg per 0.1 ml per reaction and the reaction mixtures were further incubated at room temperature for 1 hour. The mixtures were centrifuged and the cells were washed as described in Example 1. After solubilization by 0.1M NaOH, the radioactivity associated with the cells were counted in a Tricarb scillilation counter. FIG. 2 shows a competition curve between 2 to 150 ng per ml of propranolol.

The uptake of radioactive propranolol by the above reagent according to the invention can be proportionally inhibited by the native McAb. Increasing amounts of mouse ascites fluid containing the McAb were incubated with 3 H-propranolol prior to the addition of the antibodies carrying hybridoma cells. As shown in FIG. 3, the residual radioactivity bound to the cells is inversely proportional to the amount of the McAb added. This is a further proof that the uptake .sup.3 H-propranolol by the cells is an antigen-antibody reaction, inhibited both by the native antigen or by free antibodies in suspension.

EXAMPLE 4

Immunoassay for digoxin with an anti-digoxin solid phase McAb reagent according to the invention

Hybridoma Dig-18, a cloned cell line producing McAb to the drug digoxin, and NS1, the parental immunoglobulin non-secreting myeloma cell line, were fixed as described in Example 1. A constant amount of radioactive digoxin (.sup.125 I-Digoxin) was added to increasing amounts of the fixed cells and incubated at room temperature for 1 hour. The reaction mixtures were centrifuged at 3000 RPM for 20 minutes at 4.degree. C., the supernatants were aspirated and the cell pellet washed once with 1 ml cold phosphate-buffer saline. The radioactivities associated with the washed cells were measured by a Packard gamma counter. FIG. 4 shows specific binding of .sup.125 I-Digoxin to Dig-18 cells, but negligible radioactivity could be detected with the NS1 cells.

A radioimmunoassay was developed using the Dig-18-McAb preparation according to the invention. A pre-determined amount of fixed Dig-18 cells (1.times.10.sup.5 cells per 0.1 ml) was added to different dilutions of digoxin in drug-free human serum (0.1 ml). After 30 minutes at room temperature, .sup.125 I-digoxin was added to all tubes and incubated for another 30 minutes. The mixtures were centrifuged, washed and counted as described above. FIG. 5 shows a standard curve, establishing the fact that increasing concentration of unlabelled digoxin will displace the amount of .sup.125 I-digoxin bound to the solid phase non-active Dig-18-McAb reagent according to the invention.

To prove that the incorporation of .sup.125 I-digoxin to the Dig-18-McAb preparation is indeed an antigen-antibody reaction, an inhibition curve was constructed using the soluble McAb produced in vitro as culture supernatant from Clone Dig-18 to compete with the solid phase antibodies for .sup.125 I-digoxin. Results as presented in FIG. 6 indicate that increasing concentrations of the soluble McAb inhibited the incorporation of .sup.125 I-digoxin to the preparation. This result supports the notion that the McAb as presented on the solid phase reagent are indeed reacting

to the antigen in a manner competitive to the native, soluble McAb produced by the same hybridoma cell line.

EXAMPLE 5

Homogeneous Immunoassay for human serum albumin (HSA) using an anti-HSA solid phase McAb reagent according to the invention

Hybridoma B-20, a cloned cell line producing monoclonal anti-HSA antibodies; hybridoma P-49, a cell line secreting monoclonal anti-propranolol antibodies and NS1, the parental mouse myeloma cell line, were fixed by the procedure according to Example 1, except that instead of glutaraldehyde, formaldehyde at a final concentration of 10% was used as the inactivator substance.

Fixed cells of clone B-20 containing immunologically active monoclonals have been used to develop a homogeneous, non-isotopic, one-step immunoassay as described below. A total of 5.times.10.sup.5 formaldehyde-fixed hybridoma cells from clone B-20 in 50 .mu.l PBS were incubated with fluorescein-labelled HSA (FITC-HSA; 2 .mu.g HSA per 100 .mu.l per reaction, molar ratio of FITC to HSA is 3.4) at room temperature for 1 hour. The entire reaction mixture was diluted to 1 ml with PBS and analyzed by a Becton Dickinson 440 Fluorescence Activated Cell Sorter (FACS). An argon laser (300 MW, 488 nm) was used for excitation. Hybridoma fixed cells were injected at a rate of 300-800 cells per second into a 80 .mu.M PBS stream. Filters were used to permit emitted wavelength of >520 nm to be analyzed. To determine the number of positive fluorescence cells, a marker was set as a reference point to differentiate the specific stained fixed cells with that of the autofluorescence of the cells without the addition of the fluorescent conjugate. FIG. 7 shows positive staining of the majority (75%) of the population of anti-HSA McAb embedded hybridoma cells from clone B-20 (right side, solid line) by FITC-HSA, where a positively stained cell is classified as fluorescent beyond the reference point, as compared with the negative control (left side, dotted line), comprised of cells from clone B-20 and PBS alone (10% of cells above the reference point). When increasing amounts of non-labeled HSA (100 .mu.l of sample in the concentration indicated per reaction) were added to the incubation mixture, containing B-20 cells and FITC-HSA, a shift in the peak towards the negative control was observed. A total of approximately 20,000 fixed cells were analyzed in each sample. Using the reference point, the number of positive fluorescent cells was determined for each HSA concentration used (Table I). The "percent fluorescent cells" in each sample was calculated as: ##EQU1##

Incubation of FITC-HSA with fixed hybridoma cells of anti-propranolol producing clone P-49 gave no significant positive fluorescence over the unstained P-49 cells (Table II). An inhibition standard curve profile can be constructed from data obtained in Table I by calculating the relative amount of positive cells (percent bound) in each sample (FIG. 8). In the presentation of the standard curve, the value of 100% was set for the sample containing only the fixed hybridoma cells and FITC-HSA conjugate. The relative percentage of FITC-HSA bound to the cells (percent bound) in the samples with different concentrations of added HSA was calculated accordingly. Thus, the amount of antigen present in any unknown sample can be obtained via this standard competitive inhibition curve.

The above results indicate that the uptake of fluorescence of the hybridoma cells from the fluorescein labelled antigen is indeed an

immunological reaction as demonstrated by the proportional inhibition by unlabelled antigen. Furthermore, the sensitivity of the fluorescence flow cytometry together with the localized concentration of the McAb on the fixed hybridoma cells enables a direct assay of the reaction mixture without separating the excess fluorescence-labelled antigen in the sample from the "bound" fluorescence on the fixed cells. Similar results can be obtained via fluorescence microscopy.

EXAMPLE 6

One-step, non-isotopic, homogenous Immunoassay for serum propranolol

Hybridoma P-49, a cloned cell line producing monoclonal anti-propranolol antibodies, was fixed with formaldehyde as described in Example 5. A constant amount of FITC-labelled propranolol-BSA (4 nmole per 100 .mu.l per reaction, molar ratio of FITC to propranolol is 0.3) was added to 5.times.10.sup.5 fixed hybridoma cells (50 .mu.l) from clone P-49, together with human serum standard samples containing different amounts of unlabelled propranolol as indicated (100 .mu.l of serum standards per reaction). After incubation at room temperature for one hour, the entire reaction mixture is analyzed by FACS as described in Example 5. A proportional shift of the peak (representing the mean fluorescence of the majority of the cells in the sample) can be seen upon the addition of unlabelled propranolol in serum, as demonstrated by the decreasing number of fluorescent cells above the set reference gate (Table III).

These results indicate that the homogeneous, one-step immunoassay applies to small hapten molecules (such as propranolol) as well as macromolecules (such as HSA, see Example 5). Furthermore, clinical samples such as human serum can be analyzed by this direct, homogeneous method without interference to the anlytical procedure.

TABLE I

Analysis of the inhibition of fluorescent HSA attached to the B-20 fixed hybridoma cells by unlabelled HSA via Fluorescent Flow Cytometry

HSA added (.mu.g/ml)	Total No. cells analyzed	No. of cells above reference gate
0	21142	15977
1	20207	15090
5	21794	16042
10	20519	12577
25	20689	11281
50	20131	3456
100	21623	2631
200	17049	1707

TABLE II

Specific Immuno-staining of McAb embedded fixed hybridoma cells according to the invention by the correspondent FITC-antigen

"Non-active" hybridoma	FITC-HSA	Total No. of

cells used	added	No. cells analyzed	No. cells above reference gate
B-20 (anti-HSA)	2 .mu.g	20627	15842
B-20 (anti-HSA)	0	20241	3556
P-49 (anti-propranolol)	2 .mu.g	20098	1129
P-49 (anti-propranolol)	0	20629	2201

TABLE III

Homogeneous Immunoassay for serum propranolol using the solid phase McAb

Propranolol Added (.mu.M)	Total No. cells analyzed	No. cells above Reference gate
0	20030	17900
0.05	20470	15730
0.1	20370	11600
0.5	20340	9009
1	20530	7830
2	20800	6800
10	23040	3000

EXAMPLE 7

Preparation of reagents according to the invention for colorimetric test

Two procedures were successfully carried out to colour solid phase McAb reagents according to the invention. They are both outlined below.

First procedure:

1. As described in Example 1, the prepared cells were fixed with a 2.5% v/v solution of glutaldehyde and incubated for 30 minutes at 4.degree. C.
2. A 0.2% solution of trypan blue was added in a 1:1 volume and further incubated for 10 minutes.
3. The reaction mixture was centrifuged at 1200 RPM for 10 minutes at 4.degree. C. and washed with phosphate-buffered saline (PBS).
4. Glycine was added and the mixture was incubated for 10 minutes at 4.degree. C. and treated as in step No. 3.
5. The cell suspension was adjusted to 10.sup.7 cells/ml of PBS with 0.1% sodium azide and stored at 4.degree. C.

Second Procedure:

In this method the colour was added only after the cells were treated with glycine and resuspended as in step No. 5 above.

1. A 0.2% solution of trypan blue was added (1:1) to the cells and incubated for 10 minutes at 4.degree. C.
2. The reaction mixture was centrifuged at 1200 RPM for 10 minutes at 4.degree. C. and washed with PBS.
3. The cell suspension was adjusted to 10.sup.7 cells/ml of PBS with 0.1% sodium azide and stored at 4.degree. C.

Both protocols produced a cell suspension of deep blue colour and when settled showed a clear supernatant.

CLAIMS:

We claim:

1. A reagent for use in solid phase immunoassay diagnostics comprising a matrix of fixed hybridoma cells embedded with and covalently bound to its self-produced monoclonal antibodies, said bound monoclonal antibodies presenting sites available for binding to a specific antigen, and at least one auxiliary substance selected from the group consisting of labelling materials, dyes and color reagents for use in colorimetric tests.

2. A solid phase reagent according to claim 1 wherein said auxiliary substance is a member of the group consisting of dyes and colour reagents for use in colorimetric tests.

3. A solid phase reagent according to claim 1 wherein said auxiliary substance is an enzymatic labelling material.

4. A solid phase reagent according to claim 1 wherein said auxiliary substance is a fluorescent labelling material.

5. A solid phase reagent according to claim 1 wherein said auxiliary substance is a luminescent labelling material.

6. A solid phase reagent according to claim 1 wherein said auxiliary substance is a radioactive labelling material.

7. A solid phase reagent according to claim 1 mounted on a solid support.

8. A process for the preparation of a solid reagent for use in diagnostic immunoassay, comprising incubating in vitro a culture medium containing active hybridoma cells capable of producing monoclonal antibodies, allowing the formation of antibodies to proceed, separating and washing said cells free of serum, resuspending the cells in a buffer solution, adding a fixative to the resulting suspension, thus covelently binding to said hybridoma cells these self-produced monoclonal antibodies which were incapable of removal from said hybridoma cells by said washing and resuspending steps theretby to produce an immobilized fixed hybridoma cells/monoclonal antibodies complex, binding excessive free inactivator substance and isolating said immobilized fixed hybridoma cells/monoclonal antibodies complex.

9. A process according to claim 8 wherein said fixative is an aliphatic aldehyde.

10. A process according to claim 9 wherein said fixative is glutaraldehyde.

11. A process according to claim 8 wherein an auxiliary substance selected from the group consisting of labelling materials, dyes and color reagents for use in colorimetric tests is added to the reaction mixture.

12. A reagent for use in solid phase immunoassay diagnostics consisting essentially of a matrix of fixed hybridoma cells embedded with and covalently bound to its self-produced monoclonal antibodies, said bound monoclonal antibodies presenting sites available for binding to a specific antigen.

13. The reagent of claim 12, containing at least one auxiliary substance selected from the group consisting of dyes and color reagents for use in colorimetric tests and labelling materials.

ile 155:MEDLINE(R) 1966-2003/Mar W1
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Set	Items	Description

?e antiglycine

Ref	Items	Index-term
E1	1	ANTIGLYCATIVE
E2	1	ANTIGLYCEMIC
E3	3	*ANTIGLYCINE
E4	7	ANTIGLYCINERGIC
E5	1	ANTIGLYCINERGIQUES
E6	1	ANTIGLYCININ
E7	1	ANTIGLYCOALYX
E8	6	ANTIGLYCOCALICIN
E9	1	ANTIGLYCOCALYX
E10	2	ANTIGLYCODELIN
E11	2	ANTIGLYCOGEN
E12	1	ANTIGLYCOGENE

Enter P or PAGE for more

?
?s e3
S1 3 'ANTIGLYCINE'
?t s1/9/all

1/9/1
DIALOG(R)File 155:MEDLINE(R)

11264488 21293472 PMID: 11400180

Afferent synaptic contacts on glycine-immunoreactive neurons in the rat cuneate nucleus.

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Synapse (New York, N.Y.) (United States) Aug 2001, 41 (2) p139-49, ISSN 0887-4476 Journal Code: 8806914
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

This study was aimed to clarify whether the primary afferent terminals (PATs), GABAergic terminals, and glutamatergic terminals made direct synaptic contacts with glycine-IR neurons in the cuneate nucleus of rats. In this connection, injection of the anterograde tracer WGA-HRP into brachial plexus, **antiglycine** preembedding immunoperoxidase, and anti-GABA, along with antiglutamate postembedding immunogold labeling, were used to identify the PATs, glycine-IR neurons, GABA-IR terminals, and glutamate-IR terminals, respectively. The present results showed that HRP-labeled PATs, immunoperoxidase-labeled glycine-IR terminals, immunogold-labeled GABA-IR, and glutamate-IR terminals made axodendritic synaptic contacts with immunoperoxidase-labeled glycine-IR neurons. The latter three presynaptic elements also formed axosomatic synapses with glycine-IR neurons. Statistical analysis has shown that the minimum diameter of the glycine-IR dendrites postsynaptic to the above-mentioned four presynaptic elements did not differ significantly. In addition, the synaptic ratio of the glutamate-IR terminals on the glycine-IR dendrites was higher than that of GABA-IR terminals. The synaptic ratio of the GABA-IR terminals on glycine-IR dendrite was in turn higher than that of the PATs and glycine-IR terminals. It is suggested that the PATs and glutamate-IR terminals on the glycine-IR neurons may be involved in subsequent postsynaptic inhibition for spatial precision of lateral inhibition. On the other hand, the GABA-IR and glycine-IR terminals which make synaptic contacts with the dendrites of glycine-IR neurons may provide a putative means for disinhibition or facilitation to maintain the baseline neuronal activity in the rat cuneate nucleus. The results of quantitative

analysis suggest that glutamate act as the primary excitatory neurotransmitter, while GABA, when compared with glycine, may serve as a more powerful inhibitory neurotransmitter on glycine-IR neurons in the rat cuneate nucleus.

Tags: Animal; Male; Support, Non-U.S. Gov't

Descriptors: *Glycine--metabolism--ME; *Medulla Oblongata--ultrastructure --UL; *Neural Inhibition--physiology--PH; *Neurons, Afferent --ultrastructure--UL; *Presynaptic Terminals--ultrastructure--UL; *Synaptic Transmission--physiology--PH; Dendrites--metabolism--ME; Dendrites --ultrastructure--UL; Glutamic Acid--metabolism--ME; Immunohistochemistry; Medulla Oblongata--metabolism--ME; Microscopy, Electron; Neurons, Afferent --metabolism--ME; Presynaptic Terminals--metabolism--ME; Rats; Rats, Wistar ; gamma-Aminobutyric Acid--metabolism--ME

CAS Registry No.: 56-12-2 (gamma-Aminobutyric Acid); 56-40-6 (Glycine) ; 56-86-0 (Glutamic Acid)

Record Date Created: 20010611

1/9/2

DIALOG(R)File 155:MEDLINE(R)

09659824 98078803 PMID: 9418994

Morphometric study of glycine-immunoreactive neurons and terminals in the rat cuneate nucleus.

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Journal of anatomy (ENGLAND) Oct 1997, 191 (Pt 3) p375-85, ISSN 0021-8782 Journal Code: 0137162

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The distribution of glycine-immunoreactive (glycine-IR) neurons and their associated axon terminals in the rat cuneate nucleus was studied using **antiglycine** postembedding immunoperoxidase labelling and immunogold staining, respectively. The immunoperoxidase-labelled glycine-IR neurons were widely distributed in the entire rostrocaudal extent of the nucleus. They made up 30.8% (9671/31368) of the neurons surveyed. Quantitative evaluation showed that the percentage of glycine-IR neurons in the caudal level was significantly higher than that in the middle and rostral levels. The glycine-IR neurons were small cells (mean area = 198+/-1.9 microm2, n = 2862) with ovoid or spindle-shaped somata. Statistical analysis showed that the size of the glycine-IR neurons in the rostral level was significantly smaller than that in the middle and caudal levels. Immunogold labelled glycine-IR terminals which contained predominantly pleomorphic synaptic vesicles were mostly small (mean area = 1.24+/-0.03 microm2, n = 286) and they constituted 24.7% (286/1158) of the total terminals surveyed. They formed axodendritic, axosomatic and axoaxonic synapses with unlabelled elements. It is suggested from this study that glycine is one of the major neurotransmitters involved in the depression of synaptic transmission in the cuneate nucleus.

Tags: Animal; Support, Non-U.S. Gov't

Descriptors: *Glycine--metabolism--ME; *Medulla Oblongata--cytology--CY; *Medulla Oblongata--metabolism--ME; *Neurons--cytology--CY; *Neurons --metabolism--ME; Analysis of Variance; Immunoenzyme Techniques; Immunohistochemistry; Medulla Oblongata--ultrastructure--UL; Microscopy, Electron; Neurons--ultrastructure--UL; Presynaptic Terminals--ultrastructur e--UL; Rats; Rats, Inbred Strains

CAS Registry No.: 56-40-6 (Glycine)

Record Date Created: 19980202

1/9/3

DIALOG(R)File 155:MEDLINE(R)

MEDICAL DESCRIPTORS:
*drug **receptor** binding
article; high performance liquid chromatography; isotope labeling; model; stereochemistry; structure activity relation
CAS REGISTRY NO.: 483-10-3 (corynanthine); 146-48-5, 65-19-0 (yohimbine)
SECTION HEADINGS:
037 Drug Literature Index

7/9/15 (Item 5 from file: 73)
DIALOG(R)File 73:EMBASE

06134078 EMBASE No: 1995166476
Receptor **binding mimetics: A novel** molecularly imprinted polymer
Kempe M.; Mosbach K.
Dept. Pure/Applied Biochemistry, University of Lund, P.O. Box 124, S-221 00 Lund Sweden
Tetrahedron Letters (TETRAHEDRON LETT.) (United Kingdom) 1995, 36/20 (3563-3566)
CODEN: TELEA ISSN: 0040-4039
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

affinity column

A novel **molecularly imprinted polymer** was prepared by copolymerization of trimethylolpropane trimethacrylate (1) and methacrylic acid (3) in the presence of a dipeptide acting as the template. The recognition capability of the synthetic **receptor** -like binding sites produced in the polymer network for the peptide was demonstrated by using the polymer as a chiral stationary phase in HPLC. The polymer was superior to previously reported **molecularly imprinted polymers** in that unusually high racemic resolution and load capacity were demonstrated.

DRUG DESCRIPTORS:
*methacrylic acid derivative; *polymer
drug **receptor**
MEDICAL DESCRIPTORS:
*drug synthesis; *protein binding
article; high performance liquid chromatography; model
SECTION HEADINGS:
029 Clinical and Experimental Biochemistry
037 Drug Literature Index

7/9/16 (Item 6 from file: 73)
DIALOG(R)File 73:EMBASE

05724991 EMBASE No: 1994131065
Synthetic peptide receptor **mimics: Highly stereoselective recognition in non-covalent** molecularly imprinted polymers
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Tetrahedron Asymmetry (TETRAHEDRON ASYMMETRY) (United Kingdom) 1994, 5/4 (649-656)
CODEN: TASYE ISSN: 0957-4166
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

Ligand-**receptor** recognition has been studied in a **molecularly imprinted polymer** (MIP) prepared against N-Ac-L-Phe-L-Trp-Ome. The non-ionic non-covalent interaction based recognition was evaluated using the polymers as chiral HPLC stationary phases. Marked regio-, enantio- and diastereo- ligand selectivity were demonstrated with enantiomer separation factors of up to 17.8. The interaction of a series of related structures with the MIP **receptor** site has provided insight concerning its nature and